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Clinical Significance of Anti-Annexin V Antibodies in Patients With Systemic Lupus Erythematosus

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Annexin V has a calcium-dependent binding affinity for anionic phospholipids and activated platelets, and prevents prothrombinase activity. We investigated the clinical significance of IgG anti-annexin V antibodies in patients with SLE. The study population consisted of 140 patients with SLE. Sera were examined for IgG anti-annexin V antibodies by ELISA. IgG anti-annexin V antibodies were detected in 27 of 140 patients (19%). Significantly higher incidences of arterial or venous thrombosis, intrauterine fetal loss, and prolonged activated partial thromboplastin time were found in patients with anti-annexin V antibodies than in those without anti-annexin V antibodies. Three patients with thrombosis were found not to have anticardiolipin antibodies, but to show sustained serological reactions for anti-annexin V antibodies, irrespective of prednisolone administration. These results indicated the clinical characteristics of SLE patients with anti-annexin V antibodies, and that these antibodies may be associated with the pathogenesis of thrombotic events. Am. J. Hematol. 54:209–213, 1997 © Wiley-Liss, Inc.

Key words: anti-annexin V antibodies; antiphospholipid antibodies; systemic lupus erythematosus; thrombosis

INTRODUCTION

The concept of antiphospholipid syndrome (APS) has been widely accepted [1,2]. Patients with APS can be clinically classified into two subsets: patients with primary APS who do not have any underlying collagen diseases, and patients with secondary APS based on a definite diagnosis [3,4]. In patients with secondary APS, systemic lupus erythematosus (SLE) is the most common disease. It is well known that IgG is the predominant isotype related to the presentation of clinical characteristics such as arterial or venous thrombosis and intrauterine fetal loss in patients with APS [5]. Recent studies have demonstrated the serological heterogeneity of so-called antiphospholipid antibodies, including anticardiolipin antibodies (aCL) and lupus anticoagulants (LA) [6]. A portion of aCL, which is defined as phospholipid-dependent anti-β_2-glycoprotein I (β_2-GPI) antibodies, binds to an epitope appearing on conformationally altered β_2-GPI or clustered β_2-GPI at high density [7,8]. We reported that these phospholipid-dependent anti-β_2-GPI antibodies are associated with thrombotic events and may be a serological marker in a unique subset of patients with SLE [4,9]. Moreover, other proteins, such as prothrombin, protein C, and protein S, were reported as possible antigens for so-called antiphospholipid antibodies [10,11], and therefore the term ''antiphospholipid-protein antibodies'' was proposed [6].

One of the reported antigen candidates is annexin V, which was previously reported as placental anticoagulant I and was a member of the lipocorcin family [12,13]. Annexin V has a high calcium-dependent binding affinity for negatively charged phospholipids and blood platelets, and shows in vitro anticoagulant effects [12,14,15]. It was primarily reported that anti-annexin V antibodies were detected in sera from patients with rheumatoid arthritis (RA) [16]. Recent studies have shown that (1) anti-annexin V antibodies have LA properties [17], (2) annexin V is involved in the LA-induced apoptosis of

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umbilical vein endothelial cells [18], and (3) the level of annexin V is reduced on placental villi in patients with APS [19]. Moreover, Matsuda et al. reported that anti-annexin V antibodies were detected in patients with SLE and patients with habitual fetal loss or preeclampsia [20,21]. These studies suggested that SLE patients with anti-annexin V antibodies were related to the spectrum of antiphospholipid syndrome. However, the clinical characteristics of patients with SLE who have anti-annexin V antibodies have not been fully elucidated.

In this study, we studied the frequency and the clinical significance of IgG anti-annexin V antibodies in patients with SLE.

PATIENTS AND METHODS

Patients

The subjects consisted of 140 Japanese patients with SLE (124 females and 16 males; mean age 34.1 ± 12.3 years), who visited Keio University Hospital from 1974 to 1993. All of them satisfied the revised criteria for the classification of SLE established by the American Rheumatism Association (ARA: American College of Rheumatology) [22].

Medical records for all patients were retrospectively reviewed.

Diagnostic criteria of thrombosis were as follows: for cerebrovascular accident or stroke, neurological signs with an anatomically consistent infarction detected by computed tomography or magnetic resonance imaging; for deep vein thrombosis, swelling and tenderness of the leg with documentation by venography; for pulmonary embolism, chest pain and breathlessness with documentation by a radionucleotide lung scan showing a ventilation-perfusion mismatch in at least one segment; for retinal vein thrombosis, documentation by funduscopic examination. Stroke in arterial thrombosis and deep vein thrombosis in venous thrombosis were the most frequent thrombotic events in our patients, 21 patients and 15, respectively.

Sera were obtained from each patient and kept at −20°C.

ELISA

Recombinant annexin V was a kind gift from Dr. Toshiaki Hirose and Dr. Kazuo Fujikawa, Department of Biochemistry and Pathology, University of Washington. Purified annexin V gave a single band of a molecular weight of 36 kD on 7.5% SDS polyacrylamide gel under reduced condition (Fig. 1).

Sera were examined for IgG anti-annexin V antibodies by the previously reported enzyme-linked immunosorbent assay (ELISA) with slight modifications [16]. Briefly, 100 μl of purified annexin V, 5 μg/ml, was coated on each well in 96-well polystyrene microtiter plates (Immulon I: Dynatech Laboratories, USA). The wells were blocked with PBS containing 3% bovine serum albumin (BSA) for 2 hr at room temperature. Preliminary experiments indicated that diluted sera at 1:800 from healthy controls showed lower backgrounds than those at 1:200 and 1:400 (Fig. 2). Therefore, the wells were incubated with 100 μl of diluted serum at 1:800 in PBS containing 1% BSA and 0.1% Tween 20 for 1 hr at room temperature. After washing with PBS containing 0.05% Tween 20, the wells were incubated with 100 μl of horse-radish peroxidase-labeled murine monoclonal IgG against human IgG (Yamasa Corp., Japan) for 1 hr at room temperature. After washing in the same manner, bound antibodies were detected by reaction with 0.3 mM tetramethylbendizine solution containing 0.003% H_2O_2 and were read at 450 nm.

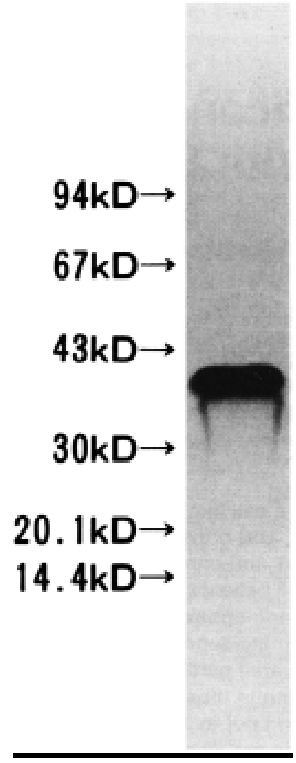

Fig. 1. SDS polyacrylamide gel electrophoresis pattern of purified annexin V. Purified annexin V gave a single band of a molecular weight of 36 kD on 7.5% SDS polyacrylamide gel under reduced condition.

The results of anti-annexin V antibody activity were expressed as units relative to the dilution of the standard serum. When antibody activity was more than 7.4 u/ml, the serum was determined to be positive. This 7.4 u/ml was the mean value plus six times the standard deviation in 45 healthy controls.

IgG aCL were screened by conventional ELISA according to the previously described method [9].

Statistical Analysis

Statistical analysis was performed using Fisher's exact test. A probability value of $P < 0.05$ was considered statistically significant.

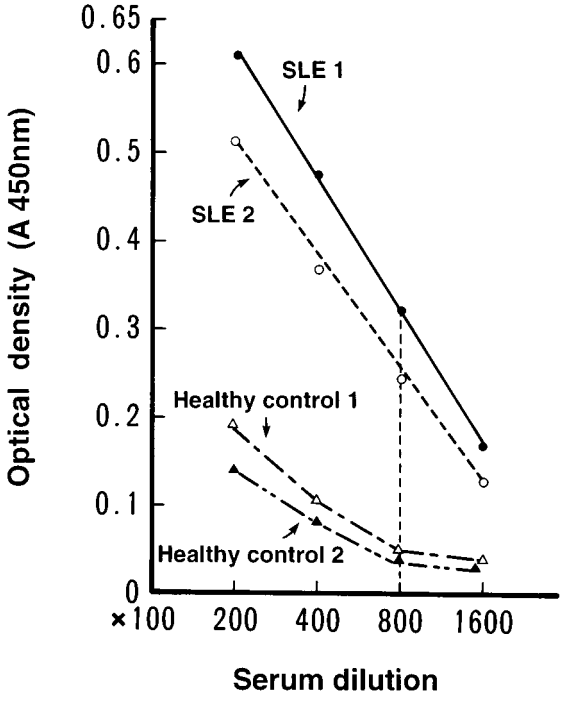

Fig. 2. Representative experiments about serial dilutions of sera in ELISA. Sera from patients with SLE showed significant binding to annexin V in ELISA. Sera from healthy controls showed lower backgrounds at the dilution of 1:800.

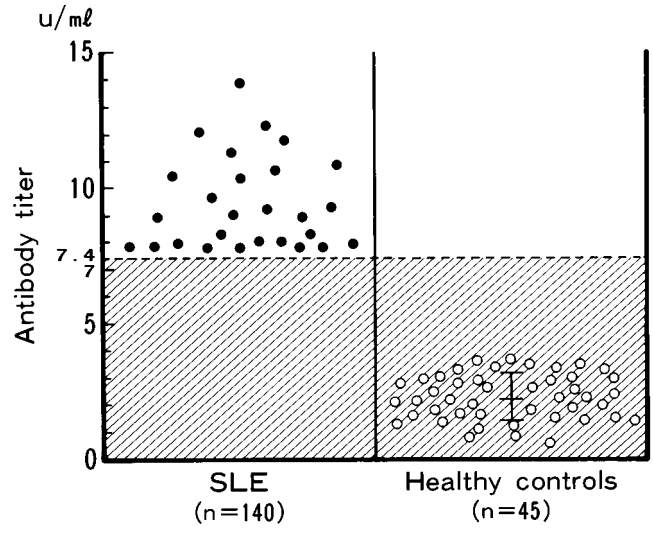

Fig. 3. Titers of IgG anti-annexin V antibodies. Titers of IgG anti-annexin V antibodies were expressed as units relative to the standard serum. The cutoff point was 7.4 u/ml, which was the mean value plus six times the standard deviation in sera from 45 healthy controls. Open circles represent individual healthy controls, whereas closed circles represent individual patients positive for IgG anti-annexin V antibodies.

RESULTS

IgG anti-annexin V antibodies were detected in 27 of 140 (19%) patients with SLE (Fig. 3). The frequency of IgG anti-annexin V antibodies were 45 of 140 (32%) and 59 of 140 (42%), respectively, if the cutoff points were determined to be the mean value plus four times and three times the standard deviation in healthy controls.

The clinical features of SLE patients with IgG anti-annexin V antibodies are summarized in Table I. The incidences of arterial or venous thrombosis, intrauterine fetal loss without any gynecological disorder in pregnant patients and prolonged activated partial thromboplastin time (APTT) were significantly higher in patients with these antibodies. The incidence of thrombocytopenia (platelet count under 10^{11}/L) was 48% (13/27) in patients with anti-annexin V antibodies and 33% (37/113) in patients without these antibodies. This difference was not significant.

IgG aCL examined by conventional ELISA were found in 61 of 140 (44%) patients with SLE [9]. Sera from 14 patients showed positive reactions for both IgG aCL and IgG anti-annexin V antibodies. The incidences of thrombosis and/or intrauterine fetal loss, which are major clinical features in patients with APS, was 52% (32/61) in patients with aCL and 44% (12/27) in patients with anti-annexin V antibodies. However, these clinical features were found in 12 of 14 (86%) patients with both aCL and anti-annexin V antibodies. This incidence is significantly ($P < 0.05$) higher than in patients with aCL or in those with anti-annexin V antibodies.

TABLE I. Clinical Characteristics in SLE Patients With IgG Anti-Annexin V Antibodies

Clinical manifestations	Positive for anti-annexin V (n = 27)	Negative for anti-annexin V (n = 113)	*P*
Arterial or venous thrombosis	12 (44%)[b]	28 (25%)	<0.05
Intrauterine fetal loss[a]	6/25 (24%)	5/99 (5%)	<0.01
Prolonged APTT	15 (56%)	23 (20%)	<0.005

[a]The incidence of intrauterine fetal loss was determined in pregnant patients.

[b]Four patients had two different sites of thrombotic events. Stroke in arterial thrombosis and deep vein thrombosis in venous thrombosis were the most frequent thrombosis in patients with anti-annexin V antibodies (8 patients and 4, respectively).

On the other hand, three patients with thrombosis were found not to have aCL, but to have anti-annexin V antibodies in their sera. Anti-annexin V antibodies showed sustained positive reactions in 5 years irrespective of the lupus activity in these patients, while they received prednisolone with daily dosage under 20 mg.

DISCUSSION

Recent advances have established the concept of APS [1,2], and have identified the heterogeneity of so-called

antiphospholipid antibodies in terms of epitope recognition [6]. Annexin V, which was termed as placental anticoagulant I [12] and was one of the lipocorcin family [13], has a high calcium-dependent binding affinity for negatively charged phospholipids and blood platelets [13,14]. Therefore, annexin V has been considered to be a protein antigen for so-called antiphospholipid antibodies. In this study, we examined the frequency of serum IgG antibodies to annexin V in patients with SLE to clarify the clinical significance of these antibodies.

The frequency of IgG anti-annexin V antibodies was 19% in our SLE patients. Matsuda et al. reported that IgG anti-annexin V antibodies were positive in 26% of their SLE patients, and that a part of binding of these antibodies was dependent on β_2-GPI [20]. One reason for the difference between our study and theirs is due to the cutoff point. They decided on the cutoff point as the mean value plus four times standard deviation in normal controls, whereas we took six times standard deviation to detect strictly anti-annexin V antibodies. The second reason is their definition of β_2-GPI-dependent anti-annexin V antibodies. These β_2-GPI-dependent anti-annexin V antibodies were defined as antibodies whose activity was enhanced in wells coated with both β_2-GPI and annexin V. However, it is important to exclude the possibility that serum polyclonal antibodies are not directed to annexin V, but also to coated β_2-GPI in their ELISA. Sammaritano et al. reported that annexin V inhibited the binding of antiphospholipid antibodies to phospholipids, but that antiphospholipid antibodies did not inhibit the binding of annexin V to phospholipids [23]. Therefore, further studies are necessary to define an antibody population which is directed to annexin V on phospholipids, and to examine the avidity of these antibodies and which portions of so-called antiphospholipid antibodies are directed to annexin V in human polyclonal serum.

Clinical characteristics in patients with anti-annexin V antibodies match the clinical features of APS such as arterial or venous thrombosis and intrauterine fetal loss [1,2]. Moreover, we found three patients with thrombosis who did not have aCL, but had anti-annexin V antibodies. Their antibody activity did not change in a 5-year follow-up period. These clinical findings suggested an important role for anti-annexin V antibodies in thrombotic events in patients with SLE. Annexin V has the in vitro anticoagulant effect by binding to negatively charged phospholipids and activated platelets [13,14], and preventing binding of activated factor X and prothrombin [15]. Thus, this phenomenon leads to a decrease in thrombin production. Further studies are necessary to examine the possibility that autoantibodies can inhibit this physiological function of annexin V, and cause thrombosis in vivo. Rand et al. found that the level of annexin V was decreased on placental villi of patients with antiphospholipid antibodies and recurrent spontaneous abortion [19]. Intrauterine fetal loss, another clinical feature of patients with anti-annexin V antibodies in our study, may be associated with their report, as there is a possibility that antibodies react to annexin V and reduce the level of annexin V, leading to a hypercoagulable state in placentae.

Prolonged APTT suggests the presence of LA activity [24]. It is well known that LA are heterogenous in terms of antibody populations [10,11]. Nakamura et al. reported that antibodies to annexin V have the properties of antiphospholipid antibodies and LA [17]. Our results were compatible with their report. LA are defined by the recently published criteria [24]. A prospective study for the detection of the activities of anti-annexin V antibodies and well-defined LA, and experiments for the separation of these antibodies will elucidate the overlapping or different features among these antibodies.

In conclusion, the frequency of IgG anti-annexin V antibodies was 19% in 140 patients with SLE. Characteristic clinical findings in these patients were thrombosis, intrauterine fetal loss, and prolonged APTT, which is also listed in the features of APS patients [1,2].

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